

AMENDMENTS TO THE CLAIMS:

Complete Listing of the Claims and Claim Status

Claims 1-169 cancelled.

170. (New) A method for obtaining a modified protein having an improved activity of interest, comprising:

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- (a) screening a library of clones to identify the presence of a clone having an activity of interest, wherein each clone of the library contains a nucleic acid obtained without selection from a mixed population of organisms from an environmental sample;
 - (b) mutagenizing one or more clones of the library;
 - (c) expressing the library to produce one or more proteins; and
 - (d) screening the proteins to identify a protein having an improved activity of interest compared to the activity identified, thereby obtaining a modified protein having an improved activity of interest.

171. (New) The method of claim 170, wherein the activity of interest is an enzymatic activity.

172. (New) The method of claim 171, wherein the enzymatic activity is provided by an esterase.

173. (New) The method of claim 171, wherein the enzymatic activity is provided by a protease.

174. (New) The method of claim 171, wherein the enzymatic activity is provided by a lipase.

175. (New) The method of claim 171, wherein the enzymatic activity is provided by a glycosidase.

176. (New) The method of claim 171, wherein the enzymatic activity is provided by a glycosyl transferase.

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177. (New) The method of claim 171, wherein the enzymatic activity is provided by a phosphatase.
 178. (New) The method of claim 171, wherein the enzymatic activity is provided by a kinase.
 179. (New) The method of claim 171, wherein the enzymatic activity is provided by a mono-oxygenase.
 180. (New) The method of claim 171, wherein the enzymatic activity is provided by a dioxygenase.
 181. (New) The method of claim 171, wherein the enzymatic activity is provided by a haloperoxidase.
 182. (New) The method of claim 171, wherein the enzymatic activity is provided by a lignin peroxidase.
 183. (New) The method of claim 171, wherein the enzymatic activity is provided by a diarylpropane peroxidase.
 184. (New) The method of claim 171, wherein the enzymatic activity is provided by an epoxide hydrolase.
 185. (New) The method of claim 171, wherein the enzymatic activity is provided by a nitrile hydratase.
 186. (New) The method of claim 171, wherein the enzymatic activity is provided by a nitrilase.
 187. (New) The method of claim 171, wherein the enzymatic activity is provided by a transaminase.
 188. (New) The method of claim 171, wherein the enzymatic activity is provided by an amidase.

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189. (New) The method of claim 171, wherein the enzymatic activity is provided by an acylase.
 190. (New) The method of claim 170, wherein the clones contain nucleic acids obtained from extremophiles.
 191. (New) The method of claim 190, wherein the extremophiles comprise thermophiles.
 192. (New) The method of claim 190, wherein the extremophiles comprise hyperthermophiles.
 193. (New) The method of claim 190, wherein the extremophiles comprise psychrophiles.
 194. (New) The method of claim 190, wherein the extremophiles comprise halophiles.
 195. (New) The method of claim 190, wherein the extremophiles comprise psychrotrophs.
 196. (New) The method of claim 190, wherein the extremophiles comprise alkalophiles.
 197. (New) The method of claim 190, wherein the extremophiles comprise acidophiles.
 198. (New) The method of claim 170, wherein the screening of (a) comprises expression screening.
 199. (New) The method of claim 170, wherein the screening of (a) comprises hybridization screening.
 200. (New) The method of claim 170, wherein the screening of (a) comprises polymerase chain reaction (PCR) screening.
 201. (New) The method of claim 170, wherein the screening of (a) comprises biopanning.
 202. (New) The method of claim 170, wherein the mutagenesis is via error-prone PCR.
 203. (New) The method of claim 170, wherein the mutagenesis is via nucleic acid shuffling.

204. (New) The method of claim 170, wherein the mutagenesis is via oligonucleotide-directed mutagenesis.
205. (New) The method of claim 170, wherein the mutagenesis is via assembly PCR.
206. (New) The method of claim 170, wherein the mutagenesis is via non-error prone PCR mutagenesis.
207. (New) The method of claim 170, wherein the mutagenesis is via *in vivo* mutagenesis.
208. (New) The method of claim 170, wherein the mutagenesis is via cassette mutagenesis.
209. (New) The method of claim 170, wherein the mutagenesis is via recursive ensemble mutagenesis.
210. (New) The method of claim 170, wherein the mutagenesis is via exponential ensemble mutagenesis.
211. (New) The method of claim 170, wherein the mutagenesis is via site-specific mutagenesis.
212. (New) The method of claim 170, wherein the mutagenesis is via ligation reassembly.
213. (New) The method of claim 170, wherein the mutagenesis is via gene site saturation mutagenesis (GSSM).
214. (New) The method of claim 170, wherein the library is generated in a prokaryotic cell.
215. (New) The method of claim 170, wherein the library is generated in a *Streptomyces* sp.
216. (New) The method of claim 215, wherein the *Streptomyces* is *Streptomyces venezuelae*.
217. (New) The method of claim 214, wherein the prokaryotic cell is gram negative.
218. (New) The method of claim 214, wherein the prokaryotic cell is a *Bacillus* sp.
219. (New) The method of claim 214 wherein the prokaryotic cell is a *Pseudomonas* sp.

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220. (New) The method of claim 170, wherein the nucleic acids are pooled prior to insertion into clones of the library.
221. (New) The method of claim 170, wherein the library is generated from pooling individual gene libraries generated from the nucleic acids.
222. (New) The method of claim 170, wherein the library comprises cDNA sequences.
223. (New) The method of claim 170, wherein the library comprises genomic sequences.
224. (New) The method of claim 170, wherein the screening of (a) is by PCR amplification of a nucleic acid sequence of interest using primers substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest, wherein the primers are labeled with a detectable molecule.
225. (New) The method of claim 170, wherein the screening of (a) is by hybridization of an oligonucleotide substantially complementary to a nucleic acid sequence of interest, wherein the oligonucleotide is labeled with a detectable molecule.
226. (New) The method of claim 170, further comprising comparing the mutated nucleic acid sequence of interest to the non-mutated nucleic acid sequence to identify the nucleotide sequence mutation.
227. (New) The method of claim 226, wherein the comparison is performed using a sequence comparison algorithm.
228. (New) The method of claim 170, wherein the screening of (a) comprises contacting a clone with a substrate wherein interaction of the substrate with the protein expressed by the clone produces a detectable signal.
229. (New) The method of claim 228, wherein the substrate comprises 5-dodecanoylamino fluorescein di-beta-D-galactopyranside (C12-FDG).

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230. (New) The method of claim 228, wherein the substrate comprises a first test protein linked to a DNA binding moiety and a second test protein linked to a transcriptional activation moiety.
231. (New) The method of claim 170, wherein the nucleic acid sequence is mutated by nucleic acid shuffling.
232. (New) The method of claim 170, wherein, prior to (b), the clones are screened for a further desired bioactivity.
233. (New) The method of claim 170, wherein the library is screened in (a) by contacting a clone of the library with a substrate, wherein a protein produced by the clone is detectable by a difference in the substrate before contact with the clone as compared to after contact.
234. (New) The method of claim 170, wherein the library is normalized before screening the library.
235. (New) The method of claim 170, wherein the nucleic acid of (a) comprises one or more open reading frames.
236. (New) The method of claim 170, wherein the protein identified in (d) is in a metabolic pathway.
237. (New) The method of claim 170, wherein the improved activity of interest comprises an enhanced or superior enzymatic activity compared to that of wild-type.
238. (New) A method for identifying a protein having an activity of interest, comprising:
(a) incubating nucleic acids obtained directly without selection from a mixed population of organisms from an environmental source with at least one oligonucleotide probe labeled with a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

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- (b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;
 - (c) generating a library from the identified nucleic acid sequences;
 - (d) screening the library for a specified activity;
 - (e) mutating a nucleic acid sequence contained in a clone from the library having the specified activity; and
 - (f) comparing the activity of an expression product of the clone from (e) following mutation with the specified activity of an expression product of the clone without mutation, wherein a difference in the is indicative of an effect of introducing at least one sequence mutation, thereby identifying a protein having an activity of interest.
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